

Sub B
1. (Amended) A [culturing] method for transfecting avian primordial germ cells [which provides for maintenance of] comprising maintaining said avian primordial germ cells for [prolonged] periods of at least fourteen days in tissue culture comprising the following steps:

(i) isolating a pure population of primordial germ cells from a desired avian; [and]

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(ii) culturing said isolated, pure population of primordial germ cells (PGCs) in a culture medium containing at least the following growth factors contained in amounts sufficient to maintain said PGCs for [prolonged periods] at least fourteen days in tissue culture:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF), for a prolonged time period; and

(iii) transfecting said avian primordial germ cells with a desired nucleic acid in order to obtain a transfected avian primordial germ cell.

2. (Amended) The method of Claim 1, wherein the minimal amounts of said growth factors [preferably range from] are:

- (1) LIF [0.1 U/ μ l to 100.0 U/ μ l] (0.00625 U/ μ l).

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- (2) bFGF [4.0 pg/μl to ~~4000 pg/μl~~] (0.25 pg/μl),
 - (3) IGF [6.0 pg/μl to ~~6000.0 pg/μl~~] (0.5625 pg/μl), and
 - (4) SCF [8.0 pg/μl to ~~8000 pg/μl~~] (4.0 pg/μl).
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7. (Amended) The method according to Claim [6] 1, wherein said PGCs are maintained in culture at least 25 days.

10. (Amended) The method of Claim [9] 1, wherein said nucleic acid sequence encodes a therapeutic peptide.

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11. (Amended) [An improved method] A method of producing chimeric avians which comprises:

- (i) isolating a pure population of primordial germ cells from an avian;
- (ii) maintaining [such] said isolated, purified PGCs in a tissue culture medium containing at least the following growth factors;
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF);
- (iii) transfecting said PGC's with a desired nucleic acid sequence;

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- (iv) [(iii)] transferring said PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs; [and]
- (v) [(iv)] allowing said recipient avian to develop into a bird; and
- (vi) [(v)] selecting for chimeric avians which [have] express the [desired PGC] phenotype conferred by the desired nucleic acid.

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15. (Amended) The method according to Claim [14] 11, wherein said nucleic acid sequence encodes a therapeutic polypeptide.

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19. (Amended) [An] A culture consisting essentially of purified isolated transfected avian PGC [cell line] cells obtained by the [culturing] method of Claim 1.

20. (Amended) The [cell line] cells of Claim [18] 19, which [is a] are chicken or turkey PGC [cell line] cells.

Kindly add the following new claims 22-26:

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22. (New) An improved method for transfecting avian primordial germ cells wherein said avian primordial germ cells are capable of being maintained for periods of at least fourteen days in tissue culture, comprising:

- (i) isolating a pure population of primordial germ cells (PGCs) from an avian;

(ii) transfecting said PGCs with a desired nucleic acid; and
(iii) maintaining said PGCs in a tissue culture medium comprising at least the following growth factors:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF);

until transfected PGCs containing the inserted nucleic acid are identified.

23. (New) The method of Claim 23, wherein said nucleic acid encodes a therapeutic polypeptide.

24. (New) The method of Claim 23, wherein said nucleic acid comprises a marker DNA, and said transfected PGCs are selected by virtue of the expression of said marker DNA.

25. (New) An improved method of producing chimeric avians which comprises:
(i) isolating a population of transfected avian primordial germ cells according to the method of Claim 22;